THE BACTERICIDAL EFFECT OF ULTRAVIOLET LIGHT ON BACTERIA IN SOME FOODS

by

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INTRODUCTION

One of the most interesting properties of ultraviolet light is its bactericidal effect. It has been known for years that this form of energy is destructive to microorganisms.

Many studies have been made of ultraviolet light and there is an immense literature on this subject. Owing to this research, germacidal lamps have been designed and are being marketed and used today. Ultraviolet lamps are available as practical sanitary aids with widespread applications and are being installed and adapted for a great diversity of uses.

In view of the general interest in the practical application of ultraviolet light, it seemed that information concerning the bactericidal effect of ultraviolet light from these lamps on bacteria in foods might be of value.

Because radiations can produce chemical or physical changes only when they are absorbed, the investigation was carried out in two parts: (1) a laboratory study to find the effective penetrating powers of ultraviolet light by using liquids of different turbidities and tissues of different thicknesses, (2) a laboratory study to determine the bactericidal power of ultraviolet light on bacteria in some foods.

REVIEW OF LITERATURE

A complete review of literature bearing upon ultraviolet light would seem out of place due to the great amount of research dealing with phases with which this study is not concerned. Therefore, an attempt will be made to limit the discussion to published results which are pertinent to this thesis, or which may have a direct bearing upon this problem.

Of the natural agencies associated with health and cleanliness, sunlight has been regarded by the majority of persons as the most beneficial. A heretic Pharaoh more than three thousand years ago insisted that the sun is god and it is the source of life and goodness. Herodotus, the prominent Egyptian physician, some 500 years later stated that sunlight is required especially for people who need restoration of muscular energy. The use of sunlight as a weapon in the treatment of disease can be traced down through the ages. After our knowledge concerning bacteria and other micro-organisms increased and their relationship with disease was proved, physicians advocated the use of sunlight in many diseases and for the destruction of organisms.

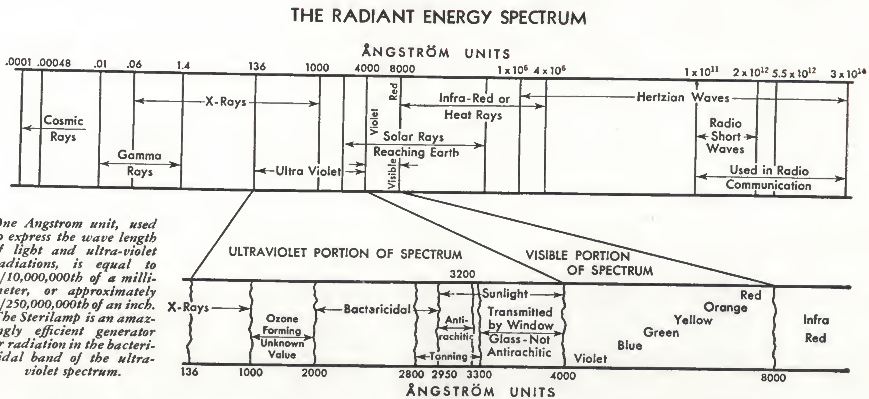
Downes and Blunt (1878) first showed that sunlight killed bacteria. Soon after this demonstration Koch (1890) showed that exposure to sunlight killed tubercle bacilli in two hours or less. In 1892, Ward, using natural sunlight, showed by actual tests that the actinic rays were responsible for the destruction of anthrax spores. About the same time and since then, various workers reported that ultraviolet light possessed

a germicidal effect.

In 1903, Barnard and Morgan noted that the germicidal action of sunlight is limited to certain of the sun's rays. Later these same workers using the spectra of carbon and other artificial sources of light for obtaining ultraviolet rays were able to kill anthrax spores. Within the past decade, the susceptibility of microorganisms to ultraviolet radiation has received renewed attention.

The visible spectrum was discovered in 1665. This visible spectrum which was thought to be one band of light, colored white, was found to be in reality a combination of many different colors of light. In addition to these bands of visible radiation, there are many forms of radiation which are invisible. X-rays, for example, are forms of invisible radiation which have the power to penetrate opaque substances. Some known as infrared rays are invisible, but this radiation is detected by the heat produced. Another form of invisible radiation is known as "ultraviolet". These forms of radiation differ in the lengths of their waves.

The ultraviolet portion of the spectrum consists of wave lengths which extend from a range of 3970 Ångström units to about 1000 Ångström units. The ultraviolet portion of the spectrum may be divided into four distinct groups. From 1000 to 2000 Ångström units are radiations which form a toxic gas, ozone, the so-called ozone-forming ultraviolet radiations. In the range from 2000 to 2950 Ångström units are to be found radiations which are most effective in destroying bacteria.



One Angstrom unit, used to express the wave length of light and ultra-violet radiations, is equal to 1/10,000,000th of a millimeter, or approximately 1/250,000,000th of an inch. The Sterilamp is an amazingly efficient generator or radiation in the bactericidal band of the ultra-violet spectrum.

Fig. 1. Spectrum charts (From The Westinghouse Sterilamp and The Rentschler-James Process of Sterilization).

Such radiations are germicidal. In the range from 2800 to 3300 Ångström units, are to be found the "biologically effective" radiations, those producing sunburn; and because they also are useful in activation of Vitamin D, they sometimes are called the antirachitic ultraviolet radiations. In the range from 3300 to 4000 Ångström units, or the region closest to the visible spectrum, are to be found radiations producing fluorescent effects. These divisions are not very sharply defined; they frequently merge and often actually overlap considerably.

Sun lamps or so-called health lamps generate waves or radiations which vary in length from 2800 to 3200 Ångström units. Accordingly they supply only long ultraviolet waves and practically of the same intensity as we get from sunlight under ordinary conditions. In fact the glass used in sun lamp bulbs will not allow the passage of radiations of wave lengths shorter than 2800 Ångström units. Sun lamps therefore do not transmit ultraviolet radiations of shorter wave lengths which are most germicidal.

All rays of the suh pass through the atmosphere before they reach the earth. The ordinary atmosphere will allow the passage of infrared rays and the rays of the visible spectrum. Most ultraviolet rays from the sun, however, fail to reach the earth. Actually the air acts as a differential filtering medium holding back many rays. In most places radiations shorter than 2900 Ångström units never penetrate to the ground; in fact the shortest to reach the ground are usually from

2950 to 3100 Ångström units in length. Less than 0.1 per cent of the total radiations from the sun is in the form of ultraviolet rays and almost all of the latter are longer than 2900 Ångström units.

There has been a great deal of investigation of the effects of ultraviolet light upon bacteria and other microorganisms using various types of mercury vapor and carbon arc lamps emitting ultraviolet light.

Salle (1943) says that in general the light rays in the ultraviolet region produce a toxic action on bacteria. The growth of an organism may be retarded or completely destroyed, depending upon the length of the rays and the period of exposure.

Sharp (1939) seeded agar plates from cultures of several species of nonspore-bearing organisms and one culture containing spores and vegetative cells of B. anthracis. Immediately after streaking, the plates were irradiated with light rays of 2537 Ångström units until the organisms were reduced to a 10 per cent survival (90 per cent killed). The results showed that spores of B. anthracis required approximately twice the exposure as vegetative cells to produce the same percentage of reduction. Herick (1937) also found that twice as much incident energy was required to destroy spores of B. megatherium as the vegetative cells.

The organism of tuberculosis (Mycobacterium tuberculosis) was irradiated by Smithburn and Lavin (1939) with sublethal doses of monochromatic light of 2537 A and found to gradually

lose their virulence and finally to become avirulent without being killed. These avirulent organisms were still capable of inducing a demonstrable immunity whereas organisms killed by the same light rays did not induce a measurable immunity. Organisms killed by light rays still possessed acid fast properties.

Spores of molds have also been treated with ultraviolet light and found to be susceptible to the same rays that are toxic to bacteria.

Viruses and bacteriophages are also sensitive to light rays. Jungeblut (1937) and Toomey (1937) found that the virus of poliomyelitis (infantile paralysis) was destroyed by light rays in the ultraviolet region. Similar results were reported by Levaditi and Voet (1935) for herpes virus and E. coli bacteriophage. Wells and Brown (1936) sprayed influenza virus into a testing chamber, followed by exposure to ultraviolet radiation. They reported that the virus was completely inactivated. Kendall and Colwell (1940) showed that bacteriophages specific for several strains of E. coli, Shigella paradysenteriae, and Staphlococcus aureus were destroyed within one minute by exposure to ultraviolet light emitted by a quartz mercury vapor lamp exposed at a distance of one cm. from the face of the lamp.

Robertson, Doyle, Tisdall, Koller, and Ward (1939) sprayed bacteria into an experimental room and showed that their spread from cubicle to cubicle was prevented by the application of ultraviolet rays of 2537 Å.

Mutations of molds (Stevens, 1930) and of bacteria (Sharp, 1940) have been produced by ultraviolet light; and Rahn (1945) said that death by ultraviolet light may well be considered to be a lethal mutation.

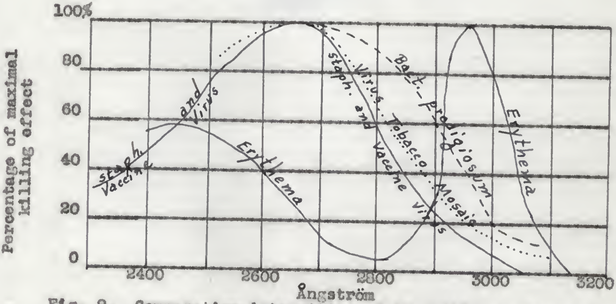


Fig. 2. Comparative intensities of the killing effect of different wavelengths acting on different organisms. (from Rahn, 1936)

He states that

> In the case of ultraviolet radiation, several million quanta must be absorbed before a cell is killed, and Wyckoff's (1932) calculation of the "sensitive zone" proved it to be only about the size of a protein molecule. Wyckoff believed that to be impossible, and considered death by ultraviolet to be quite different from that by other ways. But Gowen (cf. Duggar, 1936, p. 1323) estimated that the sensitive zone in Drosophila, which must be hit in order to produce a mutation, is about 10^{-18} cm^3, or a cube with sides of 0.01 u.
>
> This is the volume of a fairly small protein molecule. Feicke and Demerec (1937) estimated the average diameter of a gene to be about 25 Å. = 0.0025 u. Haskins and Enzmann (1936) obtained the same value. Since death of bacteria can be considered as a lethal mutation, the measurement by Wyckoff supports this viewpoint very well. The energy in a single quantum

of ultraviolet radiation seems just sufficient to inactivate the protein molecule which absorbs it, but not sufficient to cause further effects. Thus, death occurs only when an indispensable and irreplaceable protein molecule is hit by the quantum. Quanta of visible light have less energy, and cannot inactivate the protein molecule even with a direct hit, and therefore cause no death.

This simple theory of death has been questioned by Rentschler et al. (1941) who believes that "The relation between the amount of ultraviolet radiation and the per cent of bacteria killed is determined by the distribution of bacteria of different resistivity to the radiation and is not due to the probability of hitting a vital spot in a given organism by a photon." They prove quite conclusively that bacteria at the stage of rapid cell division are much more sensitive according to the method of calculation used. However, that does not disprove other experiments which were always made with resting cells. A graded resistance cannot explain the logarithmic order of death as Rahn (1943) has shown.

Luyet (1932) estimated the amount of injury by various rays upon the spores of Rhizopus nigricans by measuring the average length of mycelium per spore produced within 24 hours after exposure. He also observed spores which swelled to nearly five times their diameter, but never produced mycelium. Oster (1934) reported giant cells of yeasts and two-cell groups from three to eight times the size of normal two-cell groups, after exposure to ultraviolet radiations. Gates (1933) described a loss of cell division, but continuance of growth by E. coli after ultraviolet irradiation. Some cells continued to increase in size, especially in length, but did not divide, and produced filaments, sometimes 50 to 150 u in length, with a diameter occasionally three times normal. These cells finally degenerated, or began suddenly to divide.

Yeast cells exposed to a mercury vapor lamp lost the

ability to produce colonies on agar more rapidly than the ability to ferment sugar to alcohol and carbon dioxide (Rahn and Barnes, 1933). The cells retained after 20 minutes' exposure, 1.8 per cent of viability, 60.0 per cent fermenting capacity; after 40 minutes' exposure, 0.7 per cent viability, 39.0 per cent fermenting capacity.

Gates (1930) determined the absorption curve for ultraviolet radiations with Staphylococcus aureus and E. coli and found important points of similarity and of difference with the bactericidal curves. Ehrishmann (1930) obtained essentially the same results. The difference begins with wave lengths longer than 2800 Å. There, the great absorption is not accompanied by a corresponding death rate, probably because of the low energy per photon.

In practically all species investigated by Ehrismann (1930) the greatest absorption takes place around 2650 Å., and at this wavelength, the largest number of cells per erg of incident energy is killed. With longer and with shorter wave lengths, the percentage of killed individuals decreases. At 3300 Å., the deaths per erg are less than one per cent of this maximum and at 2400 Å., about 50 per cent of that obtained near 2650 Å.

All investigations have shown that the sensitivity of different species of bacteria varies but little. Sharp (1939) working with 10 species reports, as extreme variations of energy required to kill, 168 ergs per mm.^{2} for Shigella dysenteriae and 337 ergs for Corynebacterium diphtheria.

Even bacterial spores are easily killed. Sharp (1939) found that a culture of _B. anthracis_ with spores required 452 ergs. In 1940, he observed that a spore containing culture of _B. subtilis_ sprayed into air had to be exposed two to three times as long as _E. coli_ to obtain the same killing effect. Duggar and Hollaender (1934) could kill 85 per cent of the vegetative cells of _B. subtilis_ and _B. megatherium_ with 165 ergs per mm^2 while the spores needed 182 ergs. Lea and Haines (1940) found the spores of _B. mesentericus_ to require five times as much energy as _E. coli_.

The spores of molds are more resistant, and the resistance varies greatly with the species. Fulton and Coblentz (1929) studied the lethal action of ultraviolet radiation upon the spores of 27 widely different species. Sixteen of these could be killed by a one-minute exposure to a mercury-tungsten lamp; with four others, less than one per cent survived; but the two most resistant species had between 40 and 50 per cent survivors after a four-minute exposure. The authors explain this by "the difficulty in ray penetration of the spore walls due to their protective coloration or to their composition." The mycelium is more easily killed than the spores. The spores of _Penicillium digitatum_ required nine times as long an exposure as _E. coli_. This penicillium belonged to the 16 easily killed species; and the observation of Koller (1939) that spores of _Aspergillus niger_ require 50 to 100 times as much energy as _E. coli_ is not contradictory.

The virus of tobacco mosaic was far more resistant than the spores of B. subtilis or of B. megatherium, but responded essentially to the same wave lengths (Duggar and Hollaender, 1934).

The effective ultraviolet lamps used today to generate radiations of value for the destruction of bacteria depend upon a carefully planned type of vapor source emitting controlled and selective bactericidal ultraviolet radiations. They consume a minimum amount of power and radiate a minimum amount of heat energy. They are almost identical in operation with the fluorescent lamp, except that fluorescent powder is absent. As the current flows from electrode to electrode through mercury vapor (to which other gases are frequently added), very little visible light is produced, but most of the output of its energy is concentrated at the 2537 Ångström region of the spectrum. At least 80 per cent of the radiant energy is crowded into ultraviolet radiation at this region which provides the most effective germicidal effect (2537 Ångström units). In the case of the fluorescent lamp, the powder present on the inside is activated and fluorescence is produced. In the case of the ultraviolet lamp, the ultraviolet radiations generated pass through a special type glass used in making the lamps. This glass is purposely chosen as it will allow the passage of the selected waves of ultraviolet radiation of greatest germicidal power and at the same time absorb most of the objectionable ultraviolet wave lengths in the ozone-producing region. The low vapor

pressure and low current density in the lamp also reduce to a minimum the radiations emitted in the visible spectrum.

Equipment provided with suitable ultraviolet lamps has been designed to fill practically all needs. These lamps are marketed under different trade names, among the latter being the "Sterilamp", "Germicidal Lamp", "Safe-T-Aire Lamp", and the "Saniray". Other than keeping them clean, there is no more attention required than is necessary for an electric bulb. These lamps are available in different sizes for use in all kinds of fixtures, a fixture and ultraviolet lamp being a complete unit in itself. Under average conditions of use, they will operate for many thousands of hours, giving a continued service for at least six months (4500 hours) before replacement becomes necessary.

Wavelength in Ångström units	Energy radiated in microwatts per square centimeter at one meter
2537	26.00
2652	0.293
2804	0.027
2894	0.237
2967	0.135
3022	0.066
3129	0.510
3654	0.435
4047	0.514
4359	1.560
5461	0.850
5780	0.185

Fig. 3. Energy distribution of a "Sterilamp" operating at 40 milliamperes. (From the Westinghouse Electric and Manufacturing Company, Inc.)

TABLE OF IRRADIATION TIME FOR BACTERIA, MOLDS AND YEASTS

Clicks* on Standard Tantalum Cell Meter

BACTERIA	80-90% Kill	90-95% Kill	95-100 % Kill
Strep. homolyticus (alpha type)	15-18	18-22	22-25
Strep. hemolyticus (beta type)	15-23	23-28	28-35
Strep. lactis	25-30	30-35	35-40
Staph. aureus	15-20	20-24	24-30
Staph. albus	18-22	22-25	25-30
Neisseria catarrhalis	15-20	20-25	25-50
Micrococcus piltonensis	35-40	40-50	50-60
Micrococcus sphaeroides	50-60	60-65	65-75
Sarcina lutea	80-95	95-110	110-130
Corynebacterium diphtheriae	20-30	30-40	40-50
Shigella paradysenteriae	15-20	20-25	25-30
Eberthella typhosa	15-20	20-30	30-35
Pseudomonas fluorescens	10-20	20-25	25-32
Escherichia coli	15-20	20-25	25-30
Proteus vulgaris	10-15	15-20	20-30
Serratia marcescens	15-20	20-25	25-30
Phytomonas tumefaciens	15-20	20-25	25-45
Bacillus anthracis	20-30	30-50	50-65
Bacillus fusiformis	10-15	15-20	20-40
Bacillus subtilis	25-35	35-45	45-55
Bacillus subtilis spores	40-50	50-65	65-100
Rhodospirillum rubrum	25-30	30-35	35-40
YEASTS	**80-90% Kill**	**90-95% Kill**	**95-100% Kill**
Saccharomyces ellipsoideus (wine yeast)	20-30	30-40	40-60
Saccharomyces ellipsoideus (wild yeast from grapes)	40-50	50-60	60-80
Saccharomyces sp. (from orange juice)	25-30	30-3	35-50
Saccharomyces cerevisiae (Molasses distillery yeast)	25-35	35-40	40-60
Brewers' yeast	12-15	15-20	20-30
Bakers' yeast	10-20	20-25	25-40
Common yeast cake	20-30	30-40	40-60
MOLDS	·		**100% Kill**
Penicillium roqueforti	......	......	150
Penicillium expansum	......	......	200
Penicillium digitatum	......	......	500
Aspergillus glaucus	......	......	350
Aspergillus flavus	......	......	500
Aspergillus niger	......	......	1500
Oospora lactis	......	......	80
Mucor racemosus	......	......	180
Rhizopus nigricans	......	......	1500

** A "Click" is the amount of bactericidal ultra-violet energy produced by a Sterilamp in one second at a distance of six inches.*

Fig. 4. Table of irradiation time for bacteria, molds and yeasts. (From the Westinghouse Electric and Manufacturing Company, Inc.)

Ultraviolet rays have very little penetrating power. Rahn (1945) says that ultraviolet rays are noticeable absorbed even by such transparent substances as glass and water. Zinsser and Bayne-Jones (1939) state that ultraviolet radiations are completely absorbed by ordinary glass, by thin layers of fluids containing proteins and by tissue cells, and that they do not penetrate tissue to a depth greater than one millimeter. In the practical use of ultraviolet light as a germacide these matters must be taken into consideration.

Tanner (1944) states

> The Council of Physical Therapy of the American Medical Association recently discussed acceptance of ultraviolet lamps for disinfecting purposes. While their report concerned other situations than those which exist in the food industry, it is indirectly pertinent to it. The Council on Therapy stated that satisfactory evidence was not available to warrant acceptance of ultraviolet lamps for disinfecting solids. It was stated that the whole subject is too new, too complex, and apparently too uncertain where virulent microorganisms may be concerned. This statement would include application of ultraviolet radiation in meat-storage coolers, a much exploited application today.

The papers discussed above give an indication of the present state of knowledge concerning ultraviolet light on microorganisms. Thus it is seen that while a great amount of research has been done with ultraviolet light in the laboratory there still remains much to be learned of its practical application.

EXPERIMENTAL

Preliminary Methods and Procedures

Because radiations can produce chemical or physical changes only when they are absorbed preliminary tests were conducted in order to find the effective penetrating power of ultraviolet light by using solutions of different turbidities and by using tissues of different thicknesses.

Light Source. The ultraviolet lamp used in these tests is being marketed today by one of the leading manufacturers of electrical equipment. The electrical and physical characteristics of their available low voltage, activated electrode, germicidal lamps are given in the following tabulation

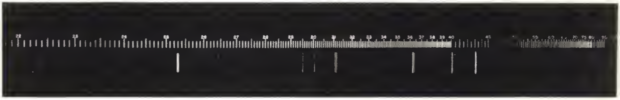
Lamp type--Watts	15
High power factor ballast--Watts	4.5
Low power factor ballast--Watts	4.5
Length overall in holders	18"
Tube diameter	1"
Glass temperature	120° F.
Glass types	972-4
Approximate lamp amperes	0.30
Approximate lamp volts	56
Average life	2500 hours

The ultraviolet lamp was installed in a cabinet which had been made for these tests. The cabinet was two feet long, one and one half feet wide and one and one fourth feet high and had a glass front and a door opening outward. The lamp was secured to the ceiling of the cabinet. Before each test, the lamp was

EXPLANATION OF PLATE I

This is a spectrograph of one of the lamps used in this study.

PLATE I



allowed to burn for 30 minutes in order that the interior of the cabinet would be as free from microorganisms as possible.

Organism Used. All of the work was conducted with a culture of E. coli. This organism was selected because it was nonspore-bearing, because of its ease of growth on most foods, because it does not hang together in chains, and because many of the previous experiments were made using this organism.

The cultures were incubated at 37° C. and were transferred to agar slants daily at a definite hour and were used after 24 hours. The organisms were washed off the agar slants, poured into a water blank, shaken thoroughly, and filtered six times through a heavy layer of absorbent cotton. The suspension was free of clumps, etc., as shown by microscopic observation and dilution tests. The number of organisms was estimated from the number of test tubes used and the appearance of turbidity. Since all steps in the procedure were well standardized, the estimate was usually accurate so that only one dilution had to be poured when plating.

Determining the Effective Penetrating Powers of Ultraviolet Light by Using Liquids of Different Turbidities. The turbid solution was prepared by adding Fullers' earth to distilled water, thoroughly agitating intermittently for an hour and then allowing it to stand 24 hours. After

standing 24 hours, the supernatant liquid was withdrawn without disturbing the sediment in the bottom. The solution was then buffered so that the pH was 7.0. Successive portions of the suspension were then diluted with distilled water until they were of the desired turbidities.

Because a turbidimeter was not available, a spectrophotometer was used for determining the turbidity of each of the solutions. The spectrophotometer was not calibrated for ultraviolet rays so in standardizing the solutions the spectrophotometer was set at 4000 Ångström units and different solutions were made allowing different percentages of the light of this wave length to pass through. The solutions were then autoclaved after which they were again checked with the spectrophotometer and brought back to the correct turbidity by adding sterile distilled water. They were then innoculated with a suspension of _E. coli_ and again checked as to amount of turbidity and reaction.

Each of these solutions was transferred to several sterile flat bottomed vials. The vials had been cut so that their depth corresponded to the inside diameter of the test tube used in standardizing them in the spectrophotometer. By filling the vials level full there were no errors due to capillary attraction. The vials were then placed at a distance of six inches from the face of the ultraviolet lamp and irradiated. At intervals of 15 seconds, one cubic centimeter was removed from a vial and plated. The plates were incubated for 48 hours at 37° C. and examined for presence

of growth. If colonies were found on the plates the solution in that particular vial had not been irradiated for a long enough time to be rendered sterile. The vial having the shortest irradiation time showing no evidence of growth when plated was taken as the one of that turbidity which had received enough energy to render it sterile.

Determining the Effective Penetrating Powers of Ultraviolet Light by Using Tissues of Different Thicknesses. Tissues were sectioned to different thicknesses by using a quick freezing microtome. Fresh beef heart was sectioned by this technic and the sections were floated over one end of short cylinders. Because sterilization by dry or moist heat caused the tissue fibers to pull apart the tissue sections on the short cylinders were sterilized by placing them in 70 per cent alcohol for 10 minutes and allowing them to dry on blotters while irradiating them with ultraviolet light. The open end of the cylinder was placed on an agar plate which had been seeded with E. coli organisms. Three cylinders each covered with a tissue of a different thickness were placed on each seeded agar plate and the plates were irradiated at a distance of six inches from the face of the ultraviolet lamp for a certain period of time. After irradiation the agar plates were incubated at 37° C. for 48 hours and examined for evidence of growth.

Preliminary Results Obtained

By using different turbidities of solutions as determined by the spectrophotometer set at 4000 Ångström units and then

irradiating _E. coli_ organisms in these solutions with an ultraviolet lamp at the distance of six inches, the following results were obtained. These results represent an average obtained by repeating the experiment a number of times and are given in the tabulated form below.

Solution allowing the passage of the following percentages of the light of 4000 Å. units	Length of time in minutes to render solution sterile
90	1 3/4
80	2
70	2
60	3 3/4
50	5½
40	15
30	20
20	90
10	150
9	Not sterile at the end of six hours

By using tissues of different thicknesses the following results were obtained. The results represent an average obtained by repeating the experiment a number of times and are given in the tabulated form below.

Time of irradiation in hours	Thickness of tissue in microns allowing sterilization
1	175
2	200
3	200
4	225
5	225
6	250

EXPLANATION OF PLATE II

This is a picture of the vials used in determining the time necessary to sterilize solutions of different turbidities. Each vial was filled with a solution of a different turbidity and placed over a typewritten number. The numbers indicate the percentage of light of the wave length of 4000 Ångström units not absorbed by the solutions as determined by a spectrophotometer.


90%
80%
70%
60%
50%
40%

EXPLANATION OF PLATE III

These two plates were seeded with a suspension of E. coli and cylinders were placed on the surface of the agar. The top end of the cylinder had been covered with a tissue of beef heart--each cylinder being covered with a tissue which had been sectioned to a different thickness. After irradiation for a definite period of time the plates were incubated at 37° C. for 48 hours and examined for growth. The thickest tissue having no growth beneath it after incubation was considered to be the thickest tissue which would allow sterilization for the period of time it had been irradiated.

Fig. A. This plate was irradiated for a period of one hour. The tissue on the left was approximately 150 microns in thickness, the one in the center 175, and the one on the right approximately 200 microns in thickness. Sterilization did not occur beneath the tissue of 200 microns in thickness as evidenced by growth.

Fig. B. This plate was irradiated for a period of six hours. The tissue on the left was approximately 250 microns in thickness and the one on the right approximately 275 microns in thickness. Sterilization did not occur beneath the tissue of 275 microns in thickness as evidenced by growth.

PLATE III

Fig. A.

Fig. B.

Methods and Procedures

<u>Light Source</u>. The same ultraviolet lamp and cabinet were used for these experiments that were used in the preliminary experiments.

<u>Foods Used</u>. The five foods used were: consumme soup, milk, canned beans, canned peas, and hamburger.

In the case of the consumme soup and milk, portions of 50 ml. of each were poured into each of two 100 ml. beakers. The top of each beaker was covered with wrapping paper and autoclaved. After autoclaving, each beaker was innoculated with one ml. of a suspension of <u>E. coli</u>. The control beaker and the test beaker were placed in the cabinet six inches from the face of the lamp. The paper was removed from the top of the test beaker but the control beaker was left covered so that no ultraviolet light could reach its contents. The beaker was irradiated for six hours.

After irradiation the contents of each beaker was transferred to a sterile water blank containing distilled water. They were diluted and plated out. The plates were incubated at 37° C. for 48 hours and counts were made.

In the case of the canned beans, canned peas, and hamburger, 50 grams of each were placed in each of two 100 ml. beakers, autoclaved, and innoculated with one ml. of <u>E. coli</u> suspension. The control beaker and the test beaker were placed in the cabinet six inches from the face of the lamp. The paper was removed from the top of the

test beaker but the control beaker was left covered so that no ultraviolet light could reach its contents. The beakers were then irradiated for six hours.

After irradiation, the contents of each beaker together with 200 ml. of sterile distilled water were transferred to a sterile Warring blender and mixed for 10 minutes. After being mixed thoroughly, one ml. was transferred to a water blank, diluted down and plated out. The plates were incubated at 37° C. for 48 hours and counts were made.

Results Obtained

After six hours' irradiation with ultraviolet light at a distance of six inches the following data were obtained.

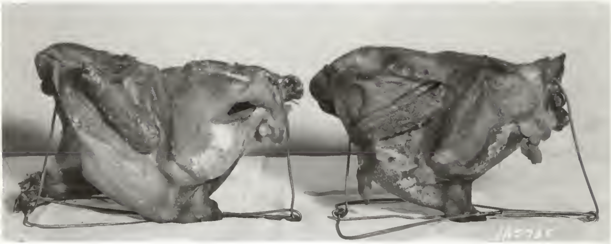
Food tested	Per cent of organisms in food after irradiation as compared with those in the non-irradiated food
Consumme soup	50
Milk	100
Canned peas	100
Canned beans	100
Hamburger	100

A number of experiments were done on the above foods following the same procedure with the exception of sterilizing the foods by autoclaving. The results were the same.

EXPLANATION OF PLATE IV

Two dressed chickens were innoculated with mold. The chicken on the left was not irradiated. The chicken on the right was irradiated at a distance of six inches from the face of the lamp. The picture was taken at the end of six days. The light colored mold growth can be seen on both chickens. However, there was no mold growing on the back of the chicken on the right where the ultraviolet light irradiated it.

PLATE IV



DISCUSSION

All tests were conducted using a distance of six inches between the face of the ultraviolet lamp and the material being irradiated. In the preliminary test after six hours' irradiation sterilization did not occur when the liquid being irradiated was of such turbidity as to prevent print being read through it. Similarly, after six hours' irradiation sterilization did not occur when beef heart tissue was used having a thickness great enough to prevent print being read through it. This indicates that the depth of sterilization achieved by ultraviolet light was less than the depth of penetration by rays of the visible spectrum.

The tests conducted with food indicated that ultraviolet irradiation had no measurable effect on organisms in food of an opaque nature. Ultraviolet irradiation had no measurable effect on organisms in liquid foods except those foods of very little turbidity, as was the case of the canned consumme soup, which allowed the ultraviolet light to penetrate enough to be effective.

CONCLUSIONS

Application of ultraviolet light as a sterilizing agent is bounded by distinct limitations. Ultraviolet radiations must be absorbed to be of any effect. A direct hit on the bacteria is necessary to kill them and ultraviolet light has very little penetrating power.

Ultraviolet light has no effect on bacteria in foods except those which possess little turbidity and will allow ultraviolet rays to penetrate.

Sterilization of most common foods by the use of ultraviolet light must be limited to the very surface.

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